Data Evaluation Report on the acute oral toxicity of Acetamiprid insecticide to Mallard Duck (Anas platyrhynchos)

PMRA Submission Numbers 99-2081, 99-2087, 99-2088, 99-2089 and 99-2090
EPA MRID Number 44651859

Data Requirement:: PMRA DATA CODE: 9.6.2.2
EPA DP Barcode:
OECD Data Point: II A 8.1.1
EPA Guideline: US EPA Subdivision E Guideline 71-1

Test material: **NI-25** **Purity (%):** >99%
Common name: Acetamiprid
Chemical name: *N*^{1}-[(6-chloro-3-pyridyl)methyl]-*N*^{2}-cyano-*N*^{1}-methylacetamidine
IUPAC: (*E*)-*N*^{1}-[(6-chloro-3-pyridyl)methyl]-*N*^{2}-cyano-*N*^{1}-methylacetamidine
CAS name: (*E*)-*N*-[(6-chloro-3-pyridinyl)methyl]-*N*'-cyano-*N*-methylethanimidamide
CAS No.: 160430-64-8
Synonyms: Pristine Brand RTU, Chipco Brand Tristar 70 WSP, Adjust Brand 70 WP and Assail Brand 70 WP

Primary Reviewer: Alison McLaughlin **Date:** January 17th 2001
For PMRA

Secondary Reviewer(s): Hemendra Mulye, PhD **Date:** June 5, 2001
{EPA/OECD/PMRA}

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CITATION: Johnson, A.J. 1994. NI-25 Acute Oral Toxicity (LD50) to the Mallard Duck, Huntingdon Research Center Ltd. (aka. Huntingdon Life Sciences Limited), Huntingdon, Cambridgeshire, England. Report No. NPS 62/932516, Sponsor: Nippon Soda Co., Tokyo, Japan. July 21 1994. Unpublished.

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EXECUTIVE SUMMARY:

The acute oral toxicity of Acetamiprid (NI-25) to 6 month old Mallard Duck (Anas platyrhynchos) was assessed over 14 days in accordance with the US EPA Avian single dose oral LD_{50} test guidelines (guideline published Oct. 1982). NI-25 was administered to groups of 5 male and 5 female adult birds, by oral intubation, at the nominal dose of 52, 73, 102, 143 or 200 mg ai/kg bw. These values were found to correspond to measured dose rates of 43, 64, 85, 124, and 181 mg ai/kg bw. LD10, LD50 and standard error values were calculated with Excel software using trend line statistics equivalent to maximum likelihood program probit analysis used by the testing facility. Based on the measured dose rate, the 14 day-acute oral LD_{50} was 87 mg a.i/kg bw (with 95% confidence limits of 65-107 mg a.i/kg bw). The 14 day NOEL of NI-25 to the Mallard Duck (Anas platyrhynchos) based on clinical signs, including abnormal behaviour and loss of co-ordination, was < 43 mg a.i/kg bw. According to the US EPA classification, Acetamiprid (NI-25) would be classified as moderately toxic to Mallard Duck (Anas platyrhynchos) on an acute oral basis.

Clinical signs of toxicity, including included subdued behaviour and the inability to stand, were observed at all test dose levels. Symptoms of intoxication were observed within 5 minutes of dosing and signs of toxicity generally increased in severity and duration with increasing dose level. A probable treatment related reduction in food consumption was observed in female birds at 143 mg/kg. Control birds remained in good health throughout the study.

This toxicity study is classified as acceptable and satisfies the guideline requirement for an acute oral toxicity study of Acetamiprid insecticide to Mallard Duck (Anas platyrhynchos).

Results Synopsis

Test Organism Size/Age (mean weight): 1176 g

LD_{50}: 84.4 mg a..i./kg bw	95% C.I.: 65 to 107 mg a.i./kg bw
LD_{10}: 17 mg a.i./kg	95% C.I.: 0 to 30 mg a.i./kg bw
NOEL: < 43 mg a.i./kg bw	Slope: 5.95 (R^2 = 0.92)

Endpoint(s) The endpoint for the LD50 and LD10 values was mortality.
Clinical toxicity was observed at the lowest dose rates of 43 mg a.i./kg bw.

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The method followed was that given in the US EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation, Wildlife and Aquatic Organisms, Series 71-1 Avian single dose oral LD_{50} test, dated Oct. 1982 and draft revised guideline dated Mar 1988.

COMPLIANCE: It was stated that this study had been conducted according to GLP Standards under OECD Principles of GLP, OECD Environment Monograph No.45, 1992 and the US EPA, FIFRA, 40 CFR Part 160, 29 November 1983/17 August 1989. It was stated that the study also complied with the GLP standards of the UK Department of Health, the EC Council Directive and the Japan Ministry of Agriculture. Signed and dated GLP, Quality Assurance, and Signature Page were provided. There was also a signed and dated Statement of No Data Confidentiality Claim.

A. MATERIALS:

1. Test Material NI-25

Description: Pale yellow powder

Lot No./Batch No. : NNI-03

Purity: >99.57 %

Stability of Compound Under Test Conditions: Results of the analytical chemistry report (Appendix 4) indicate that at nominal concentrations of 5 mg/ml, 25.3 mg/ml and 40 mg/ml, NI-25 is chemically stable in a 0.5% SCMC (sodium carboxymethylcellulose) formulation during storage

at ambient temperature for 4 hours.

Storage Conditions of Test Chemicals: Prior to testing, NI-25 was stored at 4°C in the dark; the test substance analysis certificate reported that NI-25 is stable for 1 yr in the dark at 50°C, and stable for 4 yrs in the dark at -20°C.

Physicochemical properties of NI-25.

Parameter	Values	Comments
Water solubility at 20°C	not reported	* reported elsewhere as 0.4% at 25°C
Vapour pressure	not reported	* reported elsewhere as <1.0 x 10-6 Pa ar 25°C
UV absorption	not reported	
pKa	not reported	
Kow	not reported	

* These results come from the Salinity Challenge Study in this same data submission.

2. **Test organism**:

Species: Mallard Duck (Anas platyrhynchos), 33 male and 33 female, phenotypically indistinguishable from wild birds
Age at study initiation: 6 months
Weight at study initiation: mean 1176 g (range 1035 - 1295 g) on day -15
Source: The County Game Farms, Ashford, Kent, England

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: An initial acute oral range finding study was carried out on 6 adult Mallard duck (3 males and 3 females) from HRC stock. Other details of the preliminary study were not provided.

b) Definitive Study:

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Table 1. Experimental Parameters

Parameter	**Details**	**Remarks** ***Criteria***
Acclimation Period: Conditions (same as test or not): Feeding: Health (any mortality observed):	> 3 months *conditions were the same *Food and water was offered as libitum, with the exception of 20 hour starvation period prior to dosing. *No mortality, but 2 birds were replaced prior to dosing because of unacceptably high weight loss	acceptable *EPA recommends that birds be pre-conditioned to the test facilities for at least 15 days.* *OECD recommends that birds be pre-conditioned to the test facilities for at least 7 days.*
Pen size and construction materials	Floor pens of galvanized steel with a concrete floor. Each pen measured 2.22 x 1.68 m and contained a food hopper and an automatic drinker. Straw was provided as a bedding material.	acceptable *EPA requires: pens must conform to good husbandry practices and should not create crowding stress.* *OECD lists no criteria for pen construction other than stating that pens should be suitable for the captive rearing of that species.*
Test duration	There were 15 pre-treatment days for observation, a day for dosing followed by 14 days of post treatment observation.	acceptable *EPA requires a day for dosing and at least 14 days observation.*
Dose preparation [Indicate method of confirmation of dose]	The test substance was ground into a paste with the vehicle and mixed with a high shear homogenizer.	acceptable

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Parameter	Details	Remarks *Criteria*
	Analytical results indicated that mean sample concentrations approximated the nominal concentrations.	
Mode of dose administration	Gavage - oral intubation	acceptable *Gavage or gelatin capsule.*
Dose levels Nominal (mg ai/kg bw): Measured (mg ai/kg bw):	52, 73, 102, 143, 200 43, 64, 85, 124, 181	All nominal dosages were analyzed for test substance inclusion levels. Measured concentration levels were derived from the analysis. *EPA requires a minimum of 5 treatment levels unless LD_{50} is demonstrated to be greater than 2000 mg ai/kg bw*
Solvent/vehicle, if used Type: Amount/bw:	0.5% Sodium Carboxymethylcellulose Dose rate of 5 ml/kg	acceptable *EPA recommends that the test material be administered without a vehicle if possible. Maximum vehicle concentration should not exceed 0.1 to 1.0% of body weight.*
Number of birds per groups/treatment For negative control: For solvent/vehicle control: For treated:	0 10 (5 male, 5 female) 10 (5 male, 5 female)	According to EPA guidelines, a negative control should have been included. *EPA recommends 10 birds per treatment group and 10 birds for each control and vehicle group.*
No. of feed withholding days before dosing	Food was withheld for 20 hours prior to the initiation of the test.	acceptable *EPA recommends that food should be withheld for at least 15 hours prior to dosing.*

Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Relative humidity: Photoperiod:	 14oC - 12oC 78% relative humidity 10 hr photoperiod	acceptable *EPA recommends that a 10 hr light/14 hr dark photo-period.*

b) Analytical Chemistry Report analysis for the Measured Dose Concentrations:

Table 2. Calculation of the Measured Dose

Nominal conc. (mg/ml):	Analyzed conc. (mg/ml):	Relative mean error as deviation from nominal	Analyzed conc. as % of nominal:	Nominal Dose (mg ai/kg bw)	Calculated Measured Dose (mg ai/kg bw)
10.4	8.52	-18.1 %	81.92	52	43
14.6	12.8	-12.3 %	87.67	73	64
20.4	17.1	-16.2 %	83.82	102	85
28.6	24.8	-13.3 %	86.71	143	124
40	36.2	-9.5 %	90.50	200	181

Results of the Analytical chemistry report confirmed that the dosage formulations were homogeneous and stable from the time of preparation to completion of dosing.

2. Observations:

Table 3. Observations

Parameters	Details	Remarks *Criteria*
Parameters measured		

(mortality/individual body weight at test initiation and termination/ mean feed consumption/ others)	-mortality -individual body weight -mean feed consumption -clinical observations	acceptable *EPA recommends:* *Body weight measured at test initiation, on Day 14 and at end of the test if the test is extended beyond 14 days.* *Calculation of mortality. Mortality must NOT be more than 10% in controls.* *Feed consumption may be measured as average daily food consumption.*
Indicate if the test material was regurgitated	there was no indication that the test material was regurgitated	acceptable *Regurgitation is an indication that the does was rejected. The test may have to be repeated if the problem persists.*
Groups on which necropsies were performed	-limited post mortem observations from 21 treatment related mortalities	incomplete *EPA recommends that gross necropsies be performed with inspections of the GI tract, liver, kidneys, heart, and spleen.*
Observation intervals	Clinical observations and observations of mortality were made on a daily basis. Individual body weights were recorded on days -15, -7, 0, 7, and 14. Group mean food consumption was measured for the periods between days -15 to -8, -7 to -1, 1 to 7, and 8 to 14.	Body weight observations on day 0 were made prior to dosing.
Were raw data included?	Yes, also reported as archived at the HRC testing facility	

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II. RESULTS AND DISCUSSION:

A. MORTALITY:

Following dosing, clinical signs of toxicity were observed in all treated groups, with all birds dosed with NI-25 becoming subdued and unable to stand within approximately 5 minutes of dosing. The signs of toxicity generally increased in severity and duration with increasing dose level.

Graphs were prepared for mortality versus measured dose (Appendix I) and for mortality versus nominal dose (Appendix II). The slope of the line was similar for both graphs and the R^2 correlation of mortality and dose was significant, indicating a significant dose-response effect. Rates of mortality are summarized in the following table.

Table 4: Effect of NI-25 on mortality of Mallard Duck (Anas platyrhynchos).

Measured Dose Rate (mg a.i./kg bw)	No. of birds	Distribution of mortalities				
		day 1	day 2	day 3	day 4	Total
Solvent/vehicle control	10					0
Test dose 43	10					0
Test dose 64	10	2	2			4
Test dose 85	10	3	1			4
Test dose 124	10	5	3			8
Test dose 181	10	8	2			10
LD_{10}	17 mg a.i./kg (95% C.I.: 0 to 30 mg a.i./kg bw)					
LD_{50}	84.4 mg a..i./kg bw (95% C.I.: 65 to 107 mg a.i./kg bw)					
NOEL (observed)	< 43 mg a..i./kg bw					
Slope	5.95					

R^2	0.92

B. POST MORTEM EXAMINATION RESULTS:

Limited macroscopic post mortem observations from 21 treatment related mortalities were provided. A thorough necropsy really requires histopathology to be done on the affected tissues. Indeed, even if there were no visible abnormalities (lesions), histopathology should be done on those tissues routinely sampled for toxicology, e.g., liver, kidney, heart, spleen, lung, plus intestine. Even when there are no visible (gross) abnormalities, microscopic abnormalities may be present. A path report consists of two types of description; one is a simple description of what is seen or palpated - e.g., red mucosal surface throughout all of the small intestine; the second description is a morphological diagnosis - e.g. acute haemorrhagic enteritis. From the morphological diagnosis, it is sometimes (but not always possible) to reach a conclusion about the cause of the abnormalities seen; i.e., pathological diagnosis. The brown fluid in the abdominal cavity of one bird and the red fluid in the abdominal cavity of eleven birds may be an exudate secondary to acute peritonitis although there is no description of the abdominal lining (peritoneum) to indicate that it was inflamed. There is normally a small amount of clear fluid in the abdominal and thoracic cavities, but coloured fluid suggests an exudate and an exudate suggests inflammation.

C. SUBLETHAL TOXICITY ENDPOINTS:

Clinical signs of toxicity, including included subdued behaviour and loss of co-ordination, were observed at all test dose levels. Symptoms of intoxication were observed within 5 minutes of dosing and signs of toxicity generally increased in severity and duration with increasing dose level. At the lowest dose level, where no mortality was observed, symptoms of intoxication were severe at day one but there was no record of clinical signs of toxicity during the subsequent observation period. At the higher levels of treatment, clinical signs of toxicity in surviving birds were not noted beyond day 4 following dosing.

Food consumption was variable. A probable treatment related reduction in food consumption was observed in female birds at the measured dose of 124 mg/kg (nominal dose of 143 mg/kg). Control birds remained in good health throughout the study.

Table 5: Sublethal effect of NI-25 on Mallard Duck (Anas platyrhynchos).

Measured Dose Rate (mg a.i./kg bw)		Observation							
		body weight change (g)				food consumption (g/bird/day)			
		day -15 to -8	day -7 to -1	day 1 to 7	day 8 to 14	day -15 to -8	day -7 to -1	day 1 to 7	day 8 to 14
Solvent/ vehicle control	5 male	-9	-40	+55	-22	113	129	69	57
	5 female	+77	-138	+87	0	30	163	123	106
Test dose 43	5 male	-18	-47	+11	+29	43	63	60	63
	5 female	+31	+2	-14	-2	60	94	83	109
Test dose 64	5 male	-42	+5	-15	+29	43	83	57	61
	5 female	+25	-52	+30	+58	73	86	41	129
Test dose 85	5 male	-25	-39	0	+40	50	57	52	75
	5 female	+4	-4	+45	+55	78	89	93	150
Test dose 124	5 male	-30	-32	-10	+5	43	54	43	64
	5 female	+47	-29			98	97	33	
Test dose 181	5 male	-25	-65			48	54		
	5 female	+25	-38			75	83		

C. REPORTED STATISTICS:

The proponent used probit Analysis with MLP to calculate LD_{50} values from nominal dose concentrations. The 95% confidence intervals for the LD_{50} was also reported. The reported LD_{50} value was 98 mg ai/kg bw with 95% (C.I. 81 - 119 mg ai/kg bw) based on nominal dose rates. The

slope of the dose response line was reported to be 5.95 and the standard error of slope was 1.41. The proponent did not provide graph(s) illustrating this result, nor any specific calculations showing how the results were obtained.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Results were verified in this study review using Excel software trend analysis. This type of analysis was closely equivalent to the statistical method used by the proponent. Using trend analysis, the LD_{50} value based on nominal dose concentrations was found to be 98 mg ai/kg bw (C.I. 75 - 122 mg/kg) as shown in Appendix II of this study review. This is in close agreement with the results of the proponent.

In this review, the definitive LD_{50} value was adjusted to reflect the actual dosage of a.i. which the test birds received. To this end, the LD_{50} value and the LD_{10} value were re-calculated using the measured dose concentration. The same statistical method using Excel software trend analysis was employed as shown in Appendix I of this study review. Results were as follows:

Statistical Method: Excel trend analysis on measured dosage rates (see Appendix I).

LD_{50}: 84.4 mg a..i./kg bw	95% C.I.: 65 to 107 mg a.i./kg bw
LD_{10}: 17 mg a.i./kg bw	95% C.I.: 0 to 30 mg a.i./kg bw
NOEL: < 43 mg a.i./kg bw	
Slope: 5.95	R^2: 0.92

Confidence in the projected LD_{50} and LD_{10} values is good since there was 96 % correlation between predicted and observed results (n=6).

E. STUDY DEFICIENCIES:

1) There was no negative control, however, this is unlikely to have influenced the findings of this study.

2) The proponent measured the actual concentrations of all nominal dosages, however, the values for measured concentration at each dose level were not reported. This constitutes incomplete reporting of results which necessitated re-calculation of measured concentrations for all dosages as well as generation of a new LD_{50} value on the basis of a measured rather than nominal dose.

2) Limited post mortem observations from 21 treatment related mortalities were provided. Reddened mucosa and coloured fluid were likely signs of inflammation although there was no indication as to the condition of the peritoneum. A thorough necropsy really requires histopathology to be done on the affected tissues. Indeed, even if there were no visible abnormalities, histopathology should be done on those tissues routinely sampled for toxicology, e.g., liver, kidney, heart, spleen, lung, plus intestine. The proponent should submit complete necropsy results, including histopathology results so that the biological effect of NI-25 on Mallard duck can be properly determined.

3) At the lowest dose level where no mortality was observed, symptoms of intoxication were severe at day one. There was no record of sublethal effects in the subsequent observation period, but nor was there a statement to the effect that the birds had completely recovered. The proponent should be asked to comment on the recovery of birds dosed at the nominal 52 mg ai/kg bw treatment level.

F. REVIEWER'S COMMENTS:

1) If it is possible to get a better pathology report, it would be possible to comment further on the post mortem results, but you can certainly conclude that a reddened mucosa and brown fluid in the abdomen are not normal.

G. CONCLUSIONS:

This study was deemed to be acceptable. Toxicological results were calculated on the basis of measured dosage. Statistical results were as follows:

LD_{50}: 84.4 mg a..i./kg bw	95% C.I.: 65 to 107 mg a.i./kg bw
LD_{10}: 17 mg a.i./kg bw	95% C.I.: 0 to 30 mg a.i./kg bw
NOEL: < 43 mg a.i./kg bw	

Slope: 5.95 R^2: 0.92

Following dosing, clinical signs of toxicity were observed in all treated groups, with all birds dosed with NI-25 becoming subdued and unable to stand within approximately 5 minutes of dosing. The signs of toxicity generally increased in severity and duration with increasing dose level. Limited post mortem examination results showed reddened mucosa and coloured fluid exudate in birds with treatment-related deaths. Without further post-mortem results, the physiological cause of death cannot be determined.

III. REFERENCES:

Anderson, A., Dawe, I.S., and L. Martin. Analytical Chemistry Report. NI-25: The Analysis in 0.5% Sodium Carboxymethylcellulose Formulations. NPS 62/932516.

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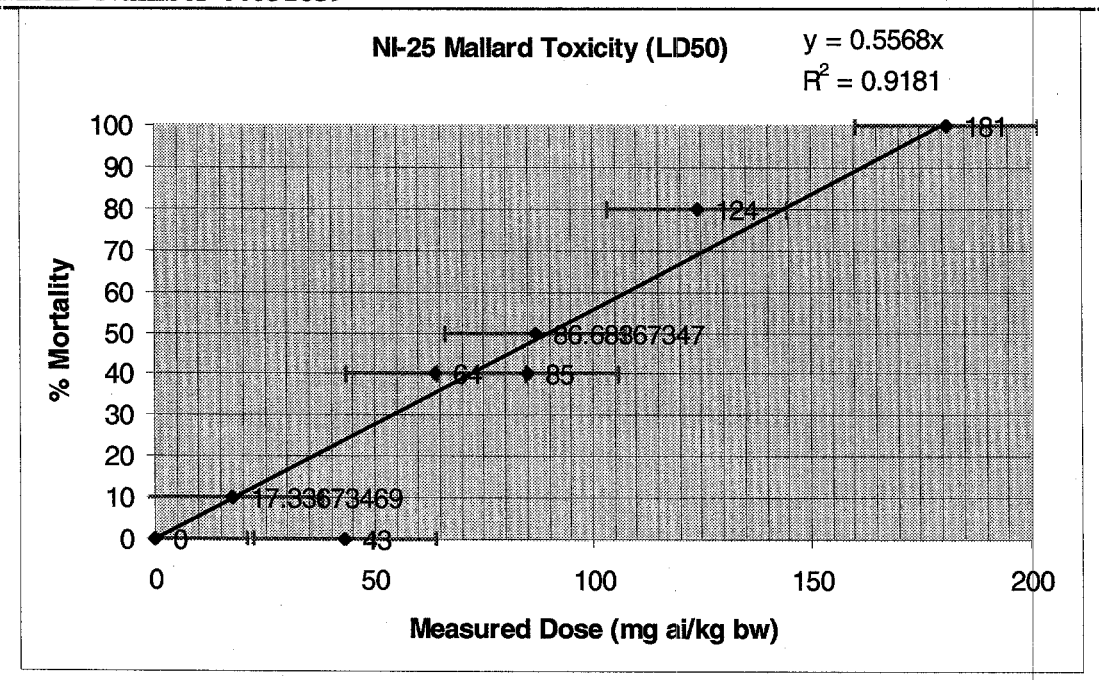
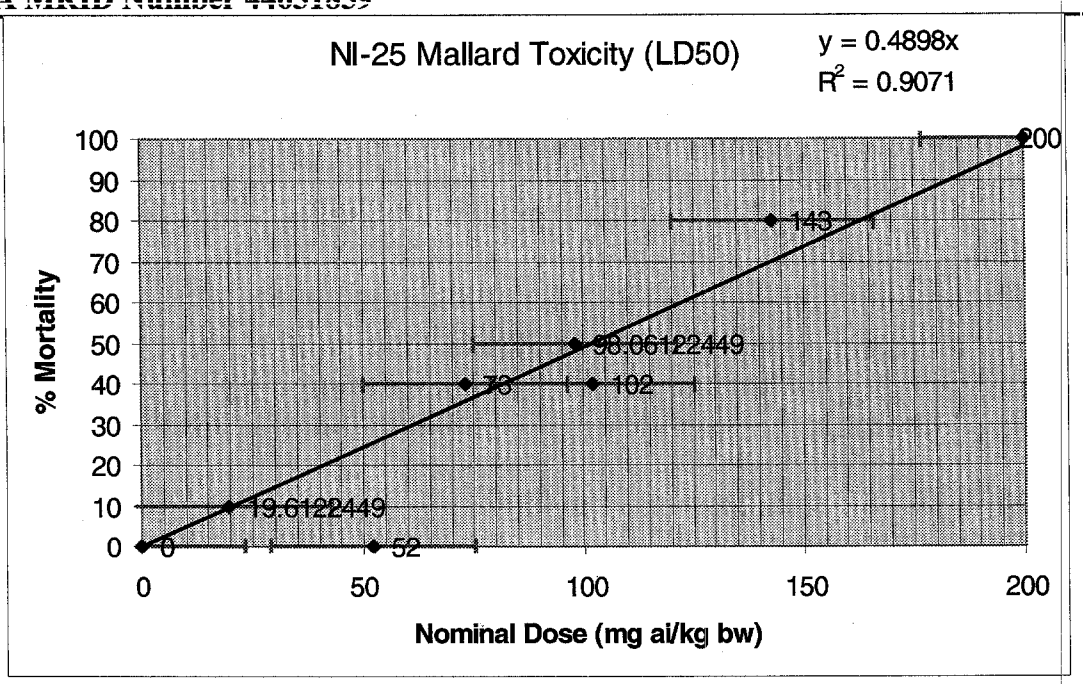


Appendix I : Values Calculated on the Basis of Measured Dosage

Predicted LD10 and LD50 values were extrapolated through a trend line Using Excel software. Standard error values were calculated and are shown in red. The equation for the slope of the line appears next to the title.

Accordingly

LD_{50}: 87 mg a..i./kg bw	**95% C.I.: 65 to 107 mg a.i./kg bw**
LD_{10}: 17 mg a.i./kg	**95% C.I.: 0 to 30 mg a.i./kg bw**



Appendix II : Values Calculated on the Basis of Nominal Dosage

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Predicted LD10 and LD50 values were extrapolated through a trend line Using Excel software. Standard error values were calculated and are shown in red. The equation for the slope of the line appears next to the title.

Accordingly

LD_{50}: 98 mg a..i./kg bw **95% C.I.: 75 to 122 mg a.i./kg bw**

LD_{10}: 20 mg a.i./kg **95% C.I.: 0 to 43 mg a.i./kg bw**